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Investigation of the technological characteristics of the *Levilactobacillus brevis strain* M3R3, isolated from Traditional Khiki Cheese, for industrial applications

Behrooz Alizadeh Behbahani[1*], Parisa Ghasemi[1], Alireza Vasiee[2], Zeinab Mousavi[1]

1- Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.
2- Department of Food Safety and Quality Control, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran.

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*Corresponding Author E-Mail:
B.alizadeh@asnrukh.ac.ir

ABSTRACT

In this research, the technological and biological characteristics of the native strain *Levilactobacillus brevis strain* M3R3, isolated from "Khiki" cheese, were comprehensively evaluated. The goal of this study was to determine the potential of this strain as an efficient starter culture and a promising probiotic for industrial applications. The results of the thermal resistance assessment showed that this strain has remarkable stability and was able to maintain acceptable cellular viability at 50 °C, 60 °C, 70 °C, and even 80 °C, with survival rates of 60.07%, 44.01%, 32.22%, and 27.81%, respectively. In the investigation of enzymatic activities, this strain demonstrated a notable ability for proteolysis by creating a hydrolysis halo with a diameter of 21.49 ± 0.13 mm, as well as high autolytic activity (51.11% at 37 °C). Furthermore, this strain was capable of producing significant amounts of exopolysaccharides (EPS), which created a mucoid appearance around the colonies. Its acidifying activity in skim milk (ΔpH24h; 1.34) was classified as a moderate acidifier. These results indicate that the *Lev. brevis* M3R3 strain is a highly suitable candidate for use in fermented dairy products and as an effective probiotic due to its resistance to thermal stress, enzymatic capabilities, and EPS production.

1- INTRODUCTION

In recent decades, significant advancements in industrial microbiology and public health have led to a growing focus on Lactic Acid Bacteria (LAB), celebrated for their probiotic and technological attributes. The surge in scientific research on these strains has resulted in a notable increase in publications [1]. This upward trend is further underscored by market projections estimating that the global probiotic market will reach $95.25 billion by 2028, highlighting their immense economic importance [2]. However, the successful industrial integration of these microorganisms depends not only on their health benefits but, more critically, on their technological robustness [3]. In this context, traditional fermented dairy products, such as Khiki cheese, serve as a unique ecological niche and a vital reservoir for isolating LAB strains with superior adaptation to harsh processing conditions [4]. Unlike commercial starter cultures, wild LAB strains isolated from these traditional matrices often possess exceptional resilience and bio-functional diversity, making them indispensable for maintaining the authentic structural and sensory profile of dairy products in modern food systems [5]. While previous studies on Iranian traditional cheeses have identified various LAB strains, there remains a critical need for isolates that possess extreme technological resilience specifically high-temperature stability which is often a limiting factor for commercial applications [6,7]

LAB are vital industrial microorganisms classified as "Generally Recognized as Safe" (GRAS). As Gram-positive, catalase-negative bacteria, their core metabolic feature is the production of lactic acid, which is key to food preservation [2]. Many LAB strains, such as Lactobacillus and Lactococcus, are recognized as probiotics with documented health benefits [8]. A prime example is *Levilactobacillus brevis*, a crucial starter culture utilized in various fermented foods [9,10]. In the production of traditional products like Khiki cheese, the indigenous LAB flora plays a dual role: ensuring microbial safety through rapid acidification and contributing to the unique ripening characteristics that define the regional product's identity [11].

The technological potential of LAB extends beyond simple fermentation. Their ability to produce exopolysaccharides (EPS) is one of their most significant attributes [12]. EPS-producing strains exhibit greater resistance to technological stresses, such as heat and mechanical shear [13]. The in situ production of these biopolymers improves viscosity and reduces undesirable syneresis, which is particularly valuable in the dairy industry for producing yogurts and cheeses with superior texture [14]. Furthermore, the enzymatic potential of LAB, specifically proteolysis and autolysis, is paramount for developing flavor and consistency [15]. In traditional cheeses, these activities accelerate the breakdown of proteins into aromatic compounds, directly influencing the final quality and consumer acceptance [16]. Notably, the synchronization of high autolytic rates with robust thermal tolerance represents a specialized technological niche that is rarely reported in common wild *Lactobacillus* isolates.

The ability of LAB to survive under diverse physicochemical conditions including varying pH, salt concentrations, and heat treatments makes them ideal candidates for food preservation [17]. Given the increasing demand for high-performance

starters, a precise evaluation of the technological characteristics of novel strains is a research necessity [18]. While general functions are documented, the efficacy of these traits varies significantly between isolates [19]. Therefore, the present study systematically evaluates the technological properties of *Levilactobacillus brevis* M3R3, specifically isolated from Khiki cheese. By characterizing its thermal resistance, EPS production, and enzymatic activities, this research aims to bridge the gap between traditional fermentation heritage and modern industrial applications, validating M3R3 as a promising candidate for the dairy industry.

2- Materials and Methods

2.1. Materials

The culture media used in this research, including MRS broth, MRS agar, and MRSC agar, were procured from Quelab (Canada). All other chemicals and reagents utilized in this study were of laboratory grade.

2.2. Probiotic Strain Activation

In this study, the probiotic strain *Lev. brevis* M3R3, which was previously isolated from local "Khiki" cheese, was utilized. The strain was maintained in a lyophilized state at the laboratory of the Food Science and Technology Department at Khuzestan University. For activation and preparation for subsequent experiments, a 100 µL aliquot of the lyophilized culture was transferred to MRS broth and incubated anaerobically at 37 °C for 48 h. Following successful activation, this culture was used to evaluate the strain's technological properties.

2.3. Thermal resistance

To evaluate thermal resistance, the *Lev. brevis* M3R3 strain was first activated by inoculating 100 µL of the stock culture into MRS broth and incubating it anaerobically for 48 h at 37 °C. Following incubation, microbial cells were harvested by centrifugation at 6,000 rpm for 5 minutes at 4 °C. The cell pellet was washed twice with PBS buffer to remove all culture medium residues before being re-suspended in the same buffer. The prepared suspensions were subsequently subjected to a heat treatment in a water bath at temperatures of 50, 60, 70, and 80 °C for 20 min. Afterward, samples were immediately incubated for 16 hat 37 °C to monitor regrowth. Finally, the level of growth was quantified by measuring the optical absorbance at 600 nm using a spectrophotometer [6].

2.4. Exopolysaccharide production

The production of exopolysaccharide (EPS) by the strain *Lev. brevis* M3R3 was investigated using a modified method based on the protocol of Ebadi Nezhad et al. (2020) [20]. For this purpose, after activating the strain, cultures were incubated anaerobically for 72 h at 37 °C on MRSC agar medium (MRS medium supplemented with 0.25% L-cysteine). To evaluate the effect of various carbon sources on EPS production, glucose, fructose, lactose, and sucrose were each added separately to the culture media at a concentration of 2%. Finally, the results of EPS production were reported visually by observing the physical characteristics of the colonies.

2.5. Acidifying ability

To evaluate the acidifying power of the strain, the method of Zaaraoui et al. (2021) was used [21]. The strain was inoculated in MRS broth and incubated for 24 h at 37 °C. Afterward, 1% of the active culture was inoculated into a sterile, reconstituted skim

milk solution (10% w/v). The pH of the samples was then measured using a pH meter after 6 and 24 h of incubation at 37 °C. The acidification rate was determined based on Equation (1):

$\Delta pH = pH_{initial} - pH_{final}$

This formula represents the difference between the final pH (pH_{final}) and the initial pH ($pH_{initial}$), indicating the extent of acidification.

2.6. Proteolysis activity

The proteolytic activity of the bacterial strain was evaluated using a spot-inoculation method on agar medium, as per the protocol of Boubakri et al. (2022) [22]. For this purpose, 100 µL of the CFS strain, prepared in PBS buffer (pH 7), was dispensed into the wells of Tryptone Soy Agar (TSA) medium supplemented with 10% (w/v) sterile skim milk. After a 24-hour incubation at 30 °C, the caseinolytic activity was determined by observing and measuring the diameter of the clear halo formed around the colonies, in millimeters. This halo indicates the degradation of milk protein (casein) by the strain's protease enzymes.

2.7. Autolytic activity

To evaluate the autolytic activity of the strain, the isolates were first cultured in MRS broth for 24 h at 37 °C. Bacterial cells were then harvested by centrifugation at 6,000 rpm for 5 min at 4 °C. The resulting cell pellets were washed twice with cold distilled water to ensure complete removal of residual culture medium. Then, the pellets were resuspended in phosphate buffer (pH 7.0) to create a cell suspension with an initial optical density (OD600) between 0.8 and 1.2. These cell suspensions were then incubated for 24 h at 4, -20, and 37 °C to monitor autolysis. The autolytic activity was quantified by calculating the percentage decrease in the OD600 value [14]. The percentage of cell autolysis was determined using the following formula (2):

$$\text{Autolysis}(\%) = \left(\frac{OD_{initial} - OD_{final}}{OD_{initial}}\right) \times 100 \quad (1)$$

where, the $OD_{initial}$ represents the initial optical density, which reflects the starting concentration of cells in the suspension. The OD_{final} is the final optical density measured after the incubation period.

2.8. Statistical analysis

Data were analyzed using One-Way Analysis of Variance (ANOVA) with SPSS statistical software (version 26) to assess the significance of observed differences. Duncan's multiple range test was used for mean comparisons. All statistical tests were conducted at a significance level of $p < 0.05$, and the experiment was performed in triplicate.

3- Results and Discussion

3.1. Thermal resistance

Thermal stability is a critical technological trait for LAB strains. This characteristic, which reflects a strain's ability to withstand high temperatures like those in industrial processes such as pasteurization, is essential for ensuring their survival during processing and maintaining viability in the final product. Thus, evaluating thermal stability serves as a crucial criterion for selecting suitable probiotic strains for use in heat-processed foods [12]. Previous studies have reported that heat-resistant strains often develop cross-resistance to other stresses, including acidic environments and high salt concentrations [23]. This suggests that cellular survival mechanisms against different stresses may overlap, and developing resistance to one type of stress can enhance tolerance to others. Based on the data from an experimental study, the thermal stability of the examined strain

significantly decreased with increasing temperature. As shown in Fig. 1, the highest cell survival rate of 60.07 ± 0.72% was recorded at 50 °C. With gradual increases in temperature to 60 °C, 70 °C, and 80 °C, the strain's survival rate decreased to 44.01 ± 0.42%, 32.22 ± 0.33%, and 27.81 ± 0.57%, respectively. The strain demonstrated considerable thermal resistance, maintaining its viability across a wide range of high temperatures. Although the cell survival rate progressively decreased with rising temperatures, the strain's ability to maintain viability highlights its inherent potential for industrial applications involving thermal stress. The findings of this study are consistent with those of Noshad et al. (2021), who assessed the thermal resistance of LAB strains isolated from native Behbahan Doogh within the temperature range of 50 °C to 80 °C [6]. Their results indicated that the *L. brevis* strain exhibited significant thermal stability. Its viability gradually decreased with increasing temperature, but the rate of decline was slower compared to other strains in their study. This strain was able to maintain viability even at 70 °C and 80 °C, demonstrating its inherent resistance to the highest tested temperatures.

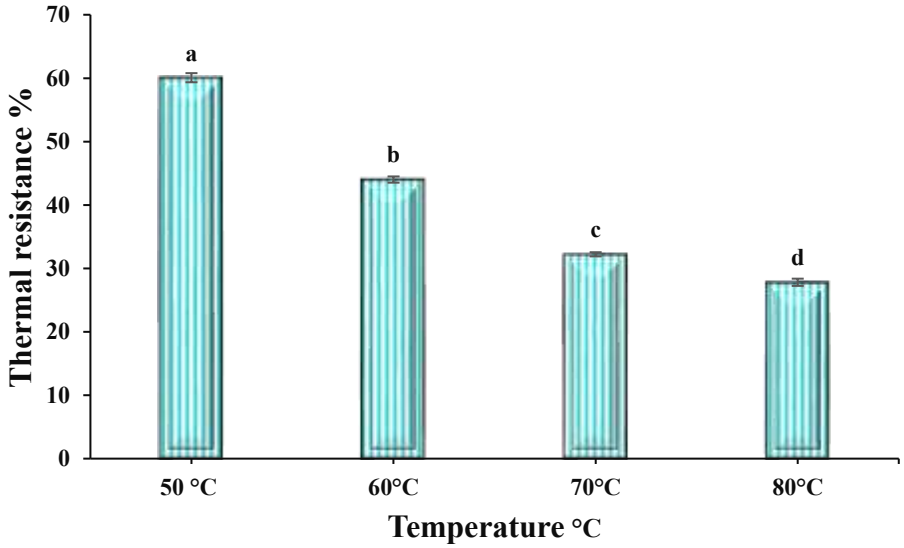

Fig. 1. Thermal resistance of *Lev. brevis* M3R3 at various temperatures (50, 60, 70 and 80 °C). Different letters above the bars indicate a statistically significant difference ($p < 0.05$).

3.2. Exopolysaccharide production

Today, LAB are garnering increasing attention for their exceptional ability to ferment a wide range of food matrices and produce high-value bioactive metabolites. Among these compounds, EPS have emerged as a key research area due to their unique technological and nutritional properties. EPS are high-molecular-weight biopolymers composed of saccharide monomeric units, displaying significant structural and functional diversity. The production and accumulation of these polymers by microorganisms typically occur under conditions of abundant carbon substrate coupled with a limitation in other essential growth elements, such as nitrogen, phosphorus, sulfur, or magnesium [24]. Based on their association with the bacterial cell, EPS are classified into different forms, including capsular or mucoid EPS (tightly bound to the cell) and released or slimy EPS

(appearing as a viscous substance in the extracellular environment). For a qualitative assessment of EPS production, the observation of mucoid colony morphology can be used as a visual indicator [19,25]. The present study revealed that the *Lev. brevis* M3R3 strain was capable of producing significant quantities of EPS, which were visible as a mucoid halo around the colonies. This phenotypic expression was directly linked to the type of carbon source present in the growth medium. As shown in Table 1, sucrose induced the most pronounced mucoid morphology (scored as +++), indicating its role as the most effective carbon substrate for EPS synthesis in this strain. Although glucose also resulted in considerable production (scored as ++), fructose and lactose yielded similar but lower EPS levels (scored as +), demonstrating a lesser impact on overall yield. These findings align with the results reported by Cheng et al. (2019), who also observed that certain LAB strains significantly increased their EPS production when grown in a sucrose-containing medium [26]. Consistent with these findings, recent studies confirm that sucrose serves as a primary signaling molecule for upregulating EPS biosynthetic genes in various *Lactobacillus* species [27]. In contrast, the study by Midik et al. (2020) suggested that glucose was the best carbon source for optimizing EPS production in various LAB strains, while fructose was shown to decrease EPS production compared to a control medium. From an industrial perspective, EPS produced by LAB play a crucial role in enhancing the physical and textural properties of fermented dairy products such as yogurt [26,28]. These biopolymers appear as filaments that interact with both bacterial cells and the casein protein network. These interactions help to stabilize the gel structure, consequently leading to an increase in the final product's viscosity [29]. Furthermore, by forming a porous structure within the yogurt matrix, EPS significantly increase the gel's water-holding capacity. This vital property prevents the undesirable phenomenon of syneresis (whey separation) and helps maintain the product's uniformity and consistency throughout storage. This porous structure also enhances the yogurt's resistance to mechanical treatments, such as stirring and pumping, preventing the breakdown of its structure during industrial processing. Therefore, the role of EPS in improving rheological properties and preventing structural defects is of paramount industrial importance [30]. Modern dairy technology now emphasizes the "in situ" use of these EPS-producing strains as a sustainable alternative to synthetic stabilizers in clean-label products [31].

Table 1. Quantitative evaluation of EPS production by *Lev. brevis* M3R3 across different carbon sources.

	Control	Lactose	Fructose	Glucose	Sucrose
Score	-	+	+	++	+++

Not. Non-mucoid (-), Weakly mucoid (+), Moderately mucoid (++), Strongly mucoid (+++).

3.3. Acidifying ability

Acidifying activity is a key characteristic of starter microorganisms, with critical applications in various industries including dairy, meat processing, plant-based products, and silage production [12]. The ability of LAB to increase acidity through fermentation not only serves as an effective

food preservation method but is also recognized as one of the most important technological properties in these industries. This trait plays a key role in the coagulation of casein in dairy products and in increasing the water-holding capacity and moisture retention during the processing and cooking of meat products [32,33]. Conversely, this property can be undesirable in certain technologies. For instance, in wine and beer production, it can lead to the formation of sour or atypical flavors and is often considered a spoilage factor [34]. In the present study, the acidifying activity of a specific strain was evaluated in a skim milk medium at 37 °C. The results showed that the strain had a notable acidifying ability, reducing the medium's pH by 0.58 ± 0.03 pH units after 6 h of incubation and by 1.34 ± 0.06 pH units after 24 h (Fig. 2). This trend indicates the strain's capacity for sustained pH reduction in the culture medium. To precisely classify the strain, we used the system proposed by Mohammed and Çon (2021) [35]. According to this criterion, strains are categorized as weak acidifiers with a $\Delta pH_{24h} \leq 1.11$, strong acidifiers with a $\Delta pH_{24h} \geq 1.61$, and moderate acidifiers with a ΔpH_{24h} ranging from 1.12 to 1.60. Based on these classifications, the studied strain, with a ΔpH_{24h} of 1.34, falls into the category of a moderate acidifier. These findings align with previous studies. For example, M'hamed et al. (2022) noted that strains with moderate acidifying ability could be used as adjunct cultures, provided they possess other important technological traits such as proteolytic and autolytic activities [36]. This acidifying ability is directly linked to the strain's specific potential to metabolize carbon and nitrogen substrates in the medium, as well as its capacity to absorb essential nutrients for growth. In contrast, Zaaraoui et al. (2021) observed a lower acidifying activity in strains isolated from goat's milk (a pH change of between 0.5 and 1.45 after 24 h) [26]. On the other hand, a study by Nachi (2019) on LAB strains isolated from wheat flour showed them to be strong acidifiers, capable of lowering the pH to below 4.0 after 24 h. The most significant factor in pH reduction is the metabolic activity of lactic acid bacteria [37]. Research by Chen et al. (2023) demonstrated that obligatory homofermentative LAB, by converting hexoses (e.g., glucose) and other organic acids (e.g., citric and malic acid) into lactic acid, cause a significant drop in pH [38]. This metabolic process was reported as the main reason for the improvement of the final product's quality. These findings were further confirmed by Cao et al. (2023), who found that LAB in fermented sausages contribute to increased stability, improved color, and enhanced product quality by maintaining a low pH [39]. This body of research clearly highlights the fundamental importance of acidifying activity in various food industries.

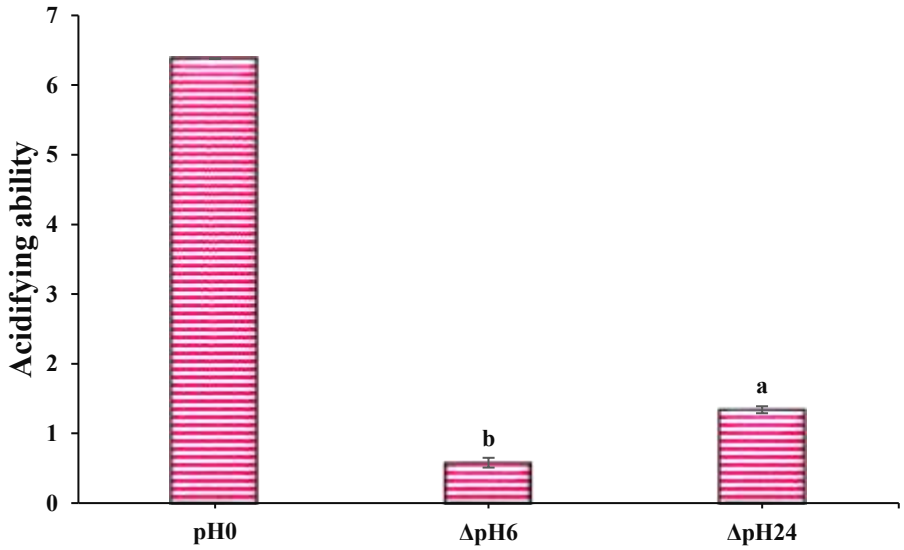

Fig.2. Acidifying activity of the strain *Lev. brevis* M3R3. The initial pH (pH0) and the amount of pH reduction after 6 h (ΔpH6) and 24 h (ΔpH24) are shown. Different letters above the bars indicate a statistically significant difference ($p < 0.05$).

3.4. Proteolysis activity

Proteolytic activity of LAB is a critical technological trait that plays a significant role in improving the organoleptic properties and texture of fermented products. This complex enzymatic system, consisting of cell membrane-bound proteinases and intracellular peptidases, breaks down complex proteins into peptides and free amino acids. These compounds not only serve as vital nitrogen sources for the growth of other fermenting bacteria but also act as flavor precursors. The catabolic process of proteolysis, along with lipase activity, leads to the creation of a wide range of volatile compounds such as esters, ketones, and lactones, which significantly enhance the sensory quality of the final product [40,41]. Recent evidence suggest that proteolytic LAB isolated from traditional sources are increasingly valued for producing functional peptides with specific health-promoting properties [42]. This process also contributes to improved protein digestibility and the release of bioactive peptides. The proteolytic activity results for the *Lev. brevis* M3R3 strain are presented in Fig. 3 As can be seen, the studied strain demonstrated a remarkable proteolytic ability in the skim milk culture medium. This activity was confirmed by the formation of a casein hydrolysis halo with an average diameter of 21.49 ± 0.13 mm. These findings underscore the high potential of this strain for the breakdown of milk proteins, making it a suitable candidate for industrial applications in dairy products. For comparison, our results are consistent with the findings of other studies but, at the same time, show stronger activity compared to some other strains. For example, Saci et al. (2025), in a study on the proteolytic potential of ten lactic acid bacteria strains isolated from Algerian Makatia goat's milk, reported clear proteolysis zones ranging from 14.33 to 32.67 mm [12]. Similarly, Fguiri et al. (2017) also evaluated the proteolytic activity of ten LAB strains isolated from camel's milk and reported hydrolysis zones in the range of 15 to 21 mm [43]. In line with previous findings, Boubakri et al. (2022) have also reported significant

proteolytic activity in lactic acid bacteria [44]. The findings of Tavakoli et al. (2019) suggest that high proteolysis can act as a key factor in increasing the survival of probiotic bacteria [45]. The proteolysis driven by lactic acid bacteria significantly improves the nutritional properties of sourdough bread. This process enriches the bread by increasing the level of free amino acids, especially lysine, which is an essential and limiting amino acid in cereals. Although traditional sourdough fermentation can lead to a dramatic increase in these amino acids, it should be noted that the bioavailability of lysine may be partially compromised during baking due to its susceptibility to the Maillard reaction [9]. This emphasizes the importance of optimizing fermentation and baking processes to preserve the maximum nutritional value of the final product. Proteolysis by lactic acid bacteria, with the production of short-chain peptides, dramatically increases the nutritional value of dairy products. These peptides have characteristics such as low allergenicity and high levels of essential amino acids. For this reason, the resulting hydrolysate can be used as a substitute for powdered milk in standardized formulations, in the production of preventive foods for individuals with cow's milk protein allergies, and as a main ingredient in beverages for regular consumption [10]. Overall, the proteolytic activity of LAB is considered a desirable and essential trait in the production of fermented foods, from dairy to grains.

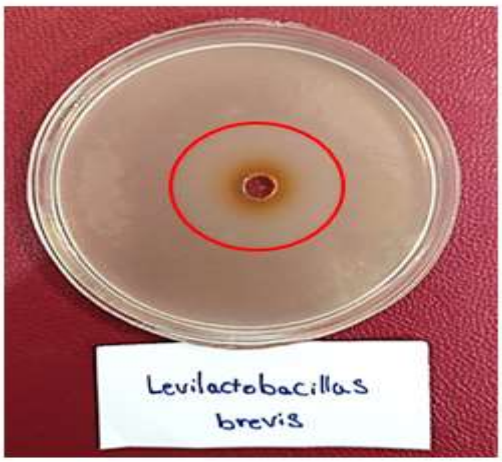

Fig. 3. Proteolytic activity of *Lev. brevis* M3R3.

3.5. Autolytic activity

Autolysis of LAB is a key technological aspect, with its importance rooted in the rapid release of intracellular enzymes. This phenomenon plays a pivotal role in post-fermentation and product ripening processes [46]. The autolytic activities of LAB strains are categorized into three groups based on their lysis rate: weak (less than 14%), moderate (15% to 24%), and good (25% to 65%) [47]. In the present study, the autolytic activity of the examined strain showed a remarkable sensitivity to temperature. As depicted in Fig. 4, the strain's autolytic rate was 51.11 ± 0.51% at 37 °C, placing it in the "good" category. This value was significantly higher than the rates observed at lower temperatures; 23.04 ± 0.12% at 4 °C and 17.32 ± 0.41% at -20 °C. Based on these data, both lower values fall into the "moderate" category, highlighting the importance of temperature control for optimizing autolysis in industrial processes. These findings align

with those of Hojjati et al. (2021), who reported a strong autolytic activity of 53% for the *Lactobacillus brevis* gp104 strain isolated from Iranian traditional cheese [7]. However, strain-specific differences were also observed; Barzegar et al. (2021) reported autolysis rates for their *L. brevis* strains ranging from 22% to 25%, placing them in the "moderate" category. This functional diversity among strains is supported by other studies [38]. For instance, Koch et al. (2008) reported a wider range of autolysis for various LAB strains, from 30.2% to 43.6%, while Cheng et al. (2022) observed a lower range of 3.61% to 8.96% for 10 different LAB strains [11,48]. This data emphasizes that the autolytic potential varies even among similar strains, underscoring the need for careful strain selection based on the intended application. In fermented dairy production, LAB autolysis is vital for determining the final product's quality. The rapid release of intracellular enzymes, particularly peptidases, accelerates the ripening process. These enzymes break down bitter peptides into amino acids, reducing the unpleasant taste in low-salt cheeses. Thus, autolysis is crucial for flavor and texture development and is considered a key factor in enhancing the sensory properties and overall quality of dairy products [39].

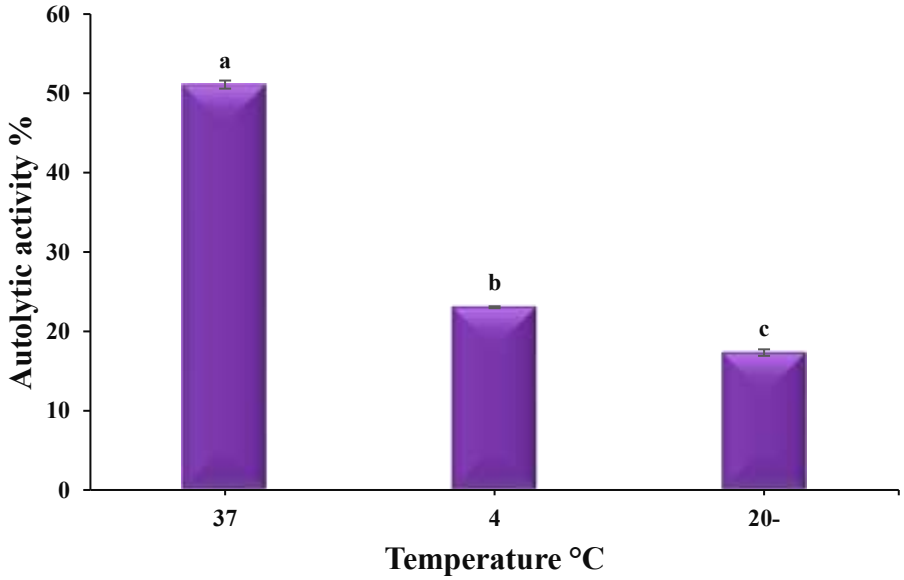

Fig. 4. Autolytic Activity of *Lev. brevis* M3R3 at Different Temperatures (37 °C, 4 °C, and -20 °C). Different letters above the bars indicate a statistically significant difference ($p < 0.05$).

3.6. Technological Comparison of *Lev. brevis* M3R3 and Commercial Starters

The comparative profiling (Table 2) reveals that *Lev. brevis* M3R3 possesses a distinct technological advantage over many commercial starters, particularly in terms of thermal resilience, autolytic potential, and EPS synthesis. While commercial cultures are often optimized for rapid acidification (Strong acidifiers) to ensure microbial safety through fast pH reduction, M3R3 exhibits a Moderate acidifying ability (ΔpH24h=1.34). This characteristic makes it an ideal adjunctive culture, as it contributes to the sensory profile and texture without causing post-acidification a common defect in fermented dairy that leads to excessive sourness and whey separation during storage.

Most notably, M3R3 demonstrates an exceptional ability to withstand temperatures up to 80 °C. This high thermal resilience is a rare and valuable trait for non-starter lactic acid bacteria (NSLAB), suggesting that the strain can survive sub-pasteurization treatments or high-temperature curd cooking in industrial cheese making. Furthermore, its 51% autolysis rate is significantly higher than the 25–50% range typical of standard industrial starters. High autolysis is critical because it triggers the early release of intracellular peptidases into the cheese matrix, which accelerates the breakdown of bitter-tasting peptides into savory free amino acids.

The synergy between this robust autolytic activity and its strong EPS production provides a dual benefit: while autolysis enhances flavor development (proteolysis-driven aromatic profiles), the EPS acts as a natural bio-thickener, stabilizing the protein network and increasing water-holding capacity. Consequently, the integration of *Lev. brevis* M3R3 into dairy formulations can dramatically accelerate the ripening process while simultaneously improving the "mouthfeel" and structural integrity of traditional and industrial cheeses, positioning it as a superior candidate for clean-label food applications.

Table 2. Comparative technological profiling of *Levilactobacillus brevis* M3R3 against commercial starters and recent industrial isolates

Technological Indicator	*Lev. brevis* M3R3	Commercial Starters	Reference/Wild Strains	Industrial Significance	Reference
Thermal Resistance	80 °C	60–75°C	70–85°C	High survival during thermal processing/sub-pasteurization	[49]
Proteolytic Activity (mm)	21.49	12–22	14.33–32.67	Enhanced flavor development and bioactive peptide release	[50]
Autolysis Degree (%)	51%	25–50	17.8–55.8	Faster ripening and reduction of bitterness in aged cheese	[51]
Acidification (ΔpH_{24h})	1.34	> 1.60	1.10–1.75	Controlled fermentation; ideal for adjunctive cultures	[52]
EPS Production	Strongly mucoid (Sucrose)	Moderate	Moderate	Improved viscosity and texture; natural bio-thickening	[53]

4- Conclusion

The results of this study confirm the multifaceted potential of the native strain *Lev. brevis strain* M3R3 for industrial and probiotic applications. Unlike previously characterized *Lev. brevis* strains from traditional Iranian sources, M3R3 exhibits a unique "technological fingerprint" marked by an exceptional resistance to

thermal stress (maintaining viability up to 80 °C) and a superior autolysis degree of 51%. A comprehensive evaluation of its key characteristics, including high enzymatic activity (proteolysis) and the ability to produce EPS, demonstrated its desirable technological properties. These traits directly contribute to improving the rheological, sensory, and nutritional qualities of fermented products and ensure the strain's survival during processing and in the gastrointestinal tract. The strong autolytic and proteolytic activities of this strain are particularly significant for accelerating cheese ripening and enhancing its flavor and texture, surpassing the performance of many common starter and non-starter LAB reported in recent literature**.** Specifically, this strain shows high potential for use as an adjunct culture in the production of traditional Iranian ripened cheeses or as a bio-thickener in industrial yogurt formulations. Furthermore, due to its remarkable thermal stability and EPS production, it could be successfully integrated into functional bakery products (e.g., sourdough) to improve texture and shelf-life. Ultimately, our findings emphasize the importance of meticulously evaluating native strains, indicating that *Lev. brevis* M3R3 is a promising candidate for use as an effective starter culture and probiotic in new, high-value-added food formulations.

Data Availability

All data relevant to the study are included in the article.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Statement

Not applicable.

Authors Contributions

Behrooz Alizadeh Behbahani: Review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Parisa Ghasemi:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Alireza Vasiee**: Writing –review & editing, Supervision, Methodology, Investigation. **Zeinab Mousavi**: Visualization, Methodology, Investigation, Formal analysis.

Consent for publication

All authors approved the manuscript for publication.

Ethical approval

This article does not contain any studies with human or animal subjects.

Clinical trial number

not applicable.

Consent to Participate declaration

not applicable.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used “Microsoft Copilot” in order to paraphrase and grammatically check the sentences. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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مقاله علمی_پژوهشی

بررسی ویژگی‌های تکنولوژیکی سویه *Levilactobacillus brevis* M3R3 جدا شده از پنیر سنتی خیکی برای کاربردهای صنعتی

بهروز علیزاده بهبهانی*[۱]، پریسا قاسمی[۱]، علیرضا وسیعی[۲]، زینب موسوی[۱]

۱- گروه علوم و مهندسی صنایع غذایی، دانشکده علوم دامی و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاثانی، ایران

۲- گروه پژوهشی ایمنی و کنترل کیفیت مواد غذایی، مؤسسه پژوهشی علوم و صنایع غذایی، مشهد، ایران

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* مسئول مکاتبات:

B.alizadeh@asnrukh.ac.ir

چکیده

در این پژوهش، ویژگی‌های تکنولوژیکی و بیولوژیکی سویه بومی *Levilactobacillus brevis* M3R3 که از پنیر «خیکی» جدا شده بود، ارزیابی شد. هدف از این مطالعه، تعیین پتانسیل این سویه به عنوان یک استارتر کارآمد و یک پروبیوتیک امیدوارکننده برای کاربردهای صنعتی بود. نتایج ارزیابی مقاومت حرارتی نشان داد که این سویه دارای پایداری قابل توجهی است و توانست در دماهای ۵۰، ۶۰، ۷۰ و حتی ۸۰ درجه سانتی‌گراد، زنده‌مانی سلولی قابل قبولی حفظ کند؛ به طوری که نرخ بقای آن به ترتیب ۶۰/۰۷، ۴۴/۰۱، ۳۲/۲۲ و ۲۷/۸۱ درصد بود. در بررسی فعالیت‌های آنزیمی، این سویه توانایی چشمگیری در پروتئولیز نشان داد و هاله هیدرولیز با قطر ۰/۱۳ ± ۲۱/۴۹ میلی‌متر ایجاد کرد؛ همچنین فعالیت اتولیتیک بالایی (۵۱/۱۱ درصد در ۳۷ درجه سانتی‌گراد) داشت. علاوه بر این، این سویه قادر به تولید مقادیر قابل توجهی اگزوپلی‌ساکارید (EPS) بود که ظاهر موکوئیدی اطراف کلنی‌ها را ایجاد می‌کرد. فعالیت اسیدی‌کننده آن در شیر بدون چربی (ΔpH۲۴h؛ ۱/۳۴) به عنوان اسیدی‌کننده متوسط طبقه‌بندی شد. این نتایج نشان می‌دهد که سویه *L. brevis* M3R3 کاندیدای بسیار مناسبی برای استفاده در محصولات لبنی تخمیری و به عنوان یک پروبیوتیک مؤثر است؛ به دلیل مقاومت در برابر تنش حرارتی، قابلیت‌های آنزیمی و تولید EPS.